

25.5.2015

MONDAY, 5/25

9:15 Present: Juuso, Anna, Tamannae

9:20 Petra, Arto arrived.

13:40 Tuukka, Tamannae and Petra arrived

14:17 Added 1 μ l DNA for AH007, AH008 and AH009

14:26 Added 1 μ l DNA for RFP-control

Transformations for propagations:

Table1

Plasmid name	Plate	Well
AH001	Plate 1	19E
AH002	Plate 3	13A
AH003	Plate 4	4G
AH004	Plate 2	2J
AH005	Plate 4	1H
AH006	Plate 3	3F
AH007	Plate 4	2H
AH008	Plate 4	6B
AH009	Plate 4	4B

Proceeded with the transformation protocol (TOP10 cells).

-> followed the protocol

14:17 Added 1 μ l DNA for AH007, AH008 and AH009

14:26 Added 1 μ l DNA for RFP-control

○ 14:36 included a H₂O control to the original RFP-control (H37) (!!!)

14:48 Moved AH007, AH008 and AH009 to heat shock (after 31 min inc) for 60s

14:50 AH007, AH008 and AH009 moved to ice for 5 mins

14:56 Moved RFP-control to heat shock

14:57 RFP-control to ice 5 mins

15:03 added 200 μ l SOC to each tube

15:08 Incubate samples 37°C 60 mins

Created new RFP-control with same protocol as earlier

((-> made double plates of each sample, one plate with 50 μ l, one with 200 μ l, and older and newer RFP-controls (#37: BBa_J04450 RFP coding device in backbone with CAM resistance) with 200 μ l

-> +37°C o/n 17 h))

Making of o/n cultures of successful transformants (AH001-AH004 and AH006) and BL21 transformation efficiency transformants.

17:00 Added 2 ml LB to six tubes (one for each transformant and one for control)

Added 1,47 ul chloramphenicol stock to each tube (25ug/ul)

Followed the protocol

17.15 Incubated with shaking overnight in 37 C

26.5.2015

TUESDAY, 5/26

MiniPreps for o/n cultures

-followed QIAprepSpin Miniprep Kit (Kit protocol can always be found with the kit)

-2ml o/n cultures - repeated step 1 twice

-1 min centrifugation in step 6

Table1

Sample	Concentration	A260/280
AH001	57,3ng/μl	1,87
AH002	38,6ng/μl	2,13
AH003	11,0ng/μl	1,91
AH004	35,0ng/μl	1,87
AH006	42,0ng/μl	
BL21 #37 control	83,0ng/μl	1,88

15.30 Anna, Petra and Juuso arrived

Propagating AH005 & AH019

- AH019 was diluted from the kit
- followed Diluting kit plasmids protocol & Transformation protocol
- AH019 was incubated on ice for 50min before heatshock of which 20 minutes without DNA

PCR to test BL21 transformants (the plasmid in them)

- Plasmid extracted with MiniPrep this morning, concentration 83,0ng/μl
 - -> use 0,5μl for as PCR template
- Use the same program and Master mix for colony PCR
 - unlike in the protocol, dilute the colony in 25μl of water (not 50μl) and use 0,5μl of treated dilution as PCR template
 - denaturation 5min, 95C
- Control sample of water (use 0,5μl)

Prepared 10μM dilutions of primers (10μl 100μM primer + 90 μl H2O)

- VF2 10μM
- VR 10 μM

Master Mix (x4)

67μl H2O (Calculate the amount of water based on how much DNA you use)

20μl 5 x Buffer

3μl 10mM dNTP mix

3μl 10μM forward primer

3μl 10μM reverse primer

2μl KAPA HiFi HotStart SNA polymerase

(0,5μl Template DNA) DO NOT INCLUDE IN THE MASTER MIX

Pipette 24,5μl of master mix into each PCR tube. Add the desired template into each tube.

1. Plasmid DNA
2. Colony DNA
3. Water control

PCR program:

95 °C 3min

98 °C 20s

57 °C 30s

72 °C 1min 30s

72 °C 5min

4 °C forever

Inoculated AH007, AH008 and AH009 in 2ml LB

AH007: 2ml LB + 1µl Ampicillin

AH008: 2ml LB + 0,8µl Kanamycin

AH009: 2ml Lb + 1,5µl Chloramphenicol

Calculations for antibiotics:

Amp

$$100000\mu\text{g/ml} \times X = 50\mu\text{g/ml} \times 2\text{ml}$$

$$(50\mu\text{g/ml} \times 2\text{ml}) / 100000\mu\text{g/ml} \\ = \underline{1\mu\text{l}}$$

Kan

$$50000\mu\text{g/ml} \times X = 20\mu\text{g/ml} \times 2\text{ml}$$

$$(20\mu\text{g/ml} \times 2\text{ml}) / 50000\mu\text{g/ml} \\ = \underline{0.8\mu\text{l}}$$

Chlor

$$34000\mu\text{g/ml} \times X = 25\mu\text{g/ml} \times 2\text{ml}$$

$$(25\mu\text{g/ml} \times 2\text{ml}) / 34000\mu\text{g/ml} \\ = 0,0014705\text{ml} \\ = \underline{1.5\mu\text{l}}$$

27.5.2015

WEDNESDAY, 5/27

MiniPreps for o/n cultures

-followed QIAprepSpin Miniprep Kit (Kit protocol can always be found with the kit)

-2ml o/n cultures - repeated step 1 twice

-1 min centrifugation in step 6

-AH007 and AH009 were mixed and contaminated with each other

->proceeded with 1ml cultures of each

Table1

Sample	Concentration	A260/280
AH007	7,9ng/μl	1,94
AH008	12,1ng/μl	1,98
AH009	8,1ng/μl	1,92

Proceeded with Restrictiong AH002, AH006, AH007 & AH008 according to Restriction digestion protocol

- Doubled the reaction volume because the DNA yields were so small
 - Pipet 5μl 10x NEB CutSmart Buffer into the tube
 - Add about 250ng DNA
 - Add 1μl of each enzyme you want to restrict with
 - Adjust total volume to 50μl with sterile water
- 31,6μl AH007 (=249,64ng)
 - 11,4μl H2O
- 6,5μl AH002 (=252,85ng)
 - 36,6μl H2O
- 20μl AH008 (242ng)
 - 23μl H2O
- 5,9μl AH006 (=247,8ng)
 - 37,1μl H2O

NOTE! There was something inside the restricted tubes, this was noted after the inactivation. Consistently in all tubes, maybe contamination?

AH005 transformants did not produce colonies, because the backbone carried ampicillin resistance instead of the chloramphenicol resistance (that we presumed it to carry) and we used chloramphenicol plates.

Ligation

Ligation of AH006 & AH007 will produce AH010 with Ampicillin resistance. The correct ligation will be 2333bp long.

Ligation of AH002 & AH008 will produce AH012 with Kanamycin resistance. The correct ligation will be 2233bp long.

For both ligations, two ligation mixes were prepared. One mix contained 2μl of the restricted DNAs (as suggested in the iGEM protocol) and the other one contained 4μl of the restricted DNAs. This was done to test the ligation efficiency. Ligation reaction was done as in Ligation protocol.

Restricting the ligations with EcoRI to run them on the gel and extract the right ligation

Restricted according to the Restriction Digestion protocol

- Used the whole ligation mix (=10μl) as our DNA sample
- Incubated in +37C for 45 minutes to ensure complete restriction but avoid start activity

○ Total restriction reaction volume 25µl

Added 5µl 6x LD into the restriction reaction after inactivation

Ran whole reactions of 1,2% agarose gel, 100V, 1h

-> we cannot separate these on the gel. Abort process.

Prepared 2ml o/n cultures from AH007, AH008, AH009, AH019

1µl Amp for AH007

0,8µl for Kan AH008

1,5µl Chlor for AH009 & AH019

-> +37C shaking

28.5.2015

THURSDAY, 5/28

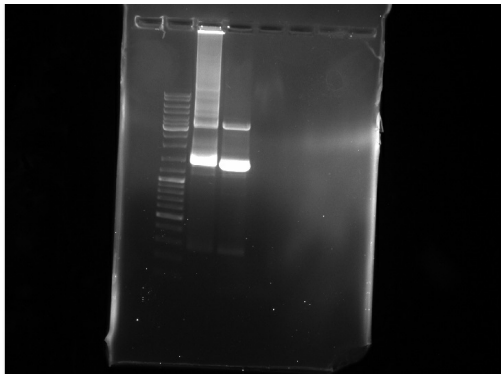
Present: Petra, Arto, Tuukka, Tamannae (until 10.15)

Made 1,2% agarose gel to run colony PCR results

Pipetted 5 ul ladder and 10 ul sample to the gel: 1: ladder, 2: PCR sample 1, 3: PCR sample 2, 4: PCR sample 3

Ran the gel in 120V, 20min

Geldoc_2015-05-28_12hr_14min.jpg



Miniprep for AH007, AH008, AH009, AH019

-followed QIAprepSpin Miniprep Kit (Kit protocol can always be found with the kit)

-2ml o/n cultures -> 2ml tube

-1 min centrifugation in step 6

Table1

Sample	Concentration (ng/μl)	A268/280
AH007	47,5	1,89
AH008	41,7	1,90
AH009	51,7	1,90
AH019	26,0	1,84

Restrictions:

We used linearized standard plasmid pSB1K3 as AH008

Proceeded with the normal protocol, DNA yields were accurate for that.

2,5μl 10x NEB CutSmart Buffer to each tube

10 ul AH008 (pSB1K3)

- 37,5 ul water

4,36 ul AH001

- 43,14 ul water

6,43 ul AH002

- 41,07ul water

22,72 ul AH003

- 24,78 ul water
- 7,14 ul AH004
- 40,36 ul water

ADD ENZYMES LAST

Finished restrictions with protocol, but added 0,25ul extra Xbal enzyme in AH006.

Ligations

Followed the ligation protocol

Ligation 1, Building 011

AH002 2µl

AH004 2µl

AH008 2µl

1µl 10x T4 DNA Ligase Buffer

3µl water

-> add 0,5µl T4 DNA Ligase

AH001 2µl

AH006 2µl

AH008 2µl

1µl 10x T4 DNA Ligase Buffer

3µl water

-> add 0,5µl T4 DNA Ligase

Ligation 2:

AH002 2µl

AH004 2µl

AH008 2µl

1µl 10x T4 DNA Ligase Buffer

3µl water

-> add 0,5µl T4 DNA Ligase

Ligation 3, building 013

AH002 2µl

AH003 2µl

AH008 2µl

1µl 10x T4 DNA Ligase Buffer

3µl water

-> add 0,5µl T4 DNA Ligase

Added T4 DNA Ligase before water

Sentrifuged 1 min 10 000 rpm before incubation

Finished ligations stored to the fridge with the code names 168, 248, 238

Transforming AH005

29.5.2015

FRIDAY, 5/29

Made a gel (1,2%) to run yesterday's ligations

Restricting the ligations with EcoRI to run them on the gel and extract the right ligation

Restricted according to the Restriction Digestion protocol

- Used the whole ligation mix (=10µl) as our DNA sample
- Incubated in +37C for 45 minutes to ensure complete restriction but avoid start activity
- Total restriction reaction volume 25µl

Added 5µl 6x LD into the restriction reaction after inactivation

Ran whole reactions of 1,2% agarose gel, 100V, 1h

Predicted lengths of plasmids:

168: 3002 bp

248: 2246 bp

238: 2248 bp

Followed Thermo-Scientific GENEJet Extraction Kit instructions, added:

168: 390 µl

248: 220 µl

238: 440 µl

of binding buffer to gel slices

followed protocol to end, results were:

AH011: 1.0 ng/µl, A260/A280 2,14

AH013: 1,4 ng/µl, A260/A280 2,04

AH014: 1,7 ng/µl, A260/A280 2,80

started ligation according to protocol, ligations incubated 30 minutes over the recommended time.

Transformed AH011, AH013 and AH014 according to the protocol. In 1 hour incubation (9.), they weren't in the shaker.

Transformed AH005 following mostly the protocol, but incubation time for the point 7 was about 2.5 minutes and for the point 9 40 minutes (20 min without shaking).

Plates were left in RT over the weekend.